The Invention

The instant invention relates to methods of screening for and identifying sequence alterations in nucleic acids. In these methods a number of nucleic acid probes are hybridized to completely complementary regions of a control nucleic acid as well as to a target nucleic acid that may or may not be completely complementary. By measuring the melting temperatures of the various probes with control and target nucleic acids, it is possible to determine if differences are present between those control and target nucleic acids. Furthermore, by using overlapping probes it is possible to use those differences in melting temperature to indicate the exact location and identity of the differences.

In one method, a plurality of overlapping probes are used to identify sequence alterations. These probes, when taken together, are an exact complement to a control nucleic acid sequence. The probes are individually hybridized to a target sequence and the melting temperature, T_m, is measured for each individual probe bound to the target. These T_ms are then compared to the T_ms of the individual probes when bound to the control sequence. The difference between the two T_ms for any one probe is termed the ΔT_m. A change in the melting temperature between the probe/control pair and the probe/target pair is indicative of a difference in sequence between the control and the target sequences. Because of the overlapping nature of the probes, there will always be a second probe that hybridizes at the site of the difference in sequence between control and the target sequences. By comparing the ΔT_m of a first probe and the ΔT_m of a second overlapping probe it is possible to determine the difference in ΔT_m ($\Delta\Delta T_m$). Because every sequence alteration will cause a distinctive change in ΔT_m, the $\Delta\Delta T_m$ can be used to indicate the location and identity of any nucleotide differences between the control and target sequences.

In another method, a plurality of nucleic acid probes, again complementary to regions of a control sequence, are hybridized to a target sequence and T_ms are measured. The difference between this method and the one described above is that a first set of nucleic acid probes complementary to regions of the control sequence separated by one or more nucleotides are used along with at least a second set of nucleic acid probes complementary to regions of the control sequence that are also separated by one or more nucleotides. Furthermore, the regions complementary to the secondary set (or sets) of probes include the nucleic acids separating the first set of probes as well as overlapping with the regions complementary to the first set of

probes. By determining the ΔT_ms and $\Delta\Delta T_m$s, as described above, one can find the location and identity of alterations between the control and target sequences

Rejection under 35 U.S.C. 112, Second Paragraph

Claims 1-16 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that the applicant regards as the invention. In particular, the Examiner finds steps c) and d) of Claims 1 and 6 vague and indefinite for failing to describe how the difference between the T_m of said target nucleic acid and said control nucleic acid is determined.

Applicants submit that steps c) and d) of Claims 1 and 6 are definite as written. Claims 1 and 6 are directed methods of identifying sequence alterations between a control nucleic acid and a target nucleic acid. As discussed above, overlapping probes are individually hybridized to a target nucleic acid and after hybridization the melting temperature of the probe-target nucleic acid is measured. This T_m is then compared to the melting temperature of an identical probe hybridized to a control nucleic acid. The difference between these two melting temperatures (T_m of the probe/target nucleic acid and T_m of the probe/control nucleic acid) is termed ΔT_m. As steps c) and d) of Claims 1 and 6 are definite as written, Applicants request withdrawal of the rejection of Claims 1-16 under 35 U.S.C. 112, second paragraph.

Rejection under 35 U.S.C. 103(a)

Claims 1-16 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Guo *et al.* (Nature Biotechnology, 1997, Vol. 15, 331-335) in view of Southern *et al.*(U.S. Patent No. 6,054,270). Applicants submit that Guo *et al.*, in view of Southern *et al.* do not render Claims 1-16 unpatentable under 35 U.S.C. 103(a).

Guo *et al.* discloses the use of the base analog 3-nitropyrrole to enhance discrimination of single nucleotide polymorphisms by artificial mismatch hybridization.

Southern *et al.* discloses a polynucleotide array that is used to analyze a labeled target nucleic acid via hybridization. Essentially, the array allows one to determine which of the arrayed sequences hybridizes most strongly to the target nucleic acid.

As discussed more fully above, the instant invention is directed to methods of identifying sequence alterations between target and control nucleic acids.

When rejecting claims under 35 U.S.C. 103, the Examiner bears the burden of establishing a *prima facie* case of obviousness. *See, e.g., In re Bell*, 26 USPQ2d 1529 (Fed Cir. 1993); M.P.E.P. ' 2142. To establish a *prima facie* case, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify or combine the teachings of the reference in the manner suggested by the Examiner. *See, e.g., In re Grabiak*, 226 USPQ2d 870 (Fed Cir. 1985). Second, the skilled artisan, in light of the teachings of the prior art, must have a reasonable expectation that the modification or combination suggested by the examiner would be successful. *See, e.g., In re Dow*, 5 USPQ2d 1529, 1531-32 (Fed Cir. 1988). Finally, the prior art reference, or references when combined, must teach or suggest each and every limitation of the rejected claims. *See, e.g.*, M.P.E.P. § 706.02(j). In addition, the teaching or suggestion to make the claimed invention as well as a reasonable expectation of success, must come from the prior art, not the applicant's disclosure. *In re Vaeck*, 20 USPQ2d 1438 (Fed Cir. 1991); M.P.E.P. § 706.02(j). If any one of these criteria is not met, *prima facie* obviousness is not established.

The Examiner states that one of ordinary skill in the art would have been motivated to combine the teachings of Guo *et al.* and Southern *et al.* because both disclose that mismatched nucleotides will reduce the melting temperature of a probe hybridized to a nucleic acid. The Applicants respectfully submit that this is merely a recitation of a physical fact, that melting temperature increases when there are higher levels of complementarity between two hybridized nucleic acids, and not a motivation to combine the two references. As the examiner has not pointed to any teaching in the references that would motivate one of ordinary skill in the art to combine the references, other than that both state that melting temperature will decrease when mismatches are introduced into a pair of hybridizing nucleic acids, the Examiner has not carried her burden in establishing a *prima facie* case of obviousness and the rejection under 35 U.S.C. 103(a) should be withdrawn.

The Examiner makes no statement as to whether a skilled artisan would have a reasonable expectation that the combination suggested by the examiner would be successful. As such a statement is explicitly required to establish a *prima facie* case of obviousness, the

Examiner has not carried her burden and the rejection under 35 U.S.C. 103(a) should be withdrawn.

Finally, the references, even when combined, do not teach each and every limitation of the rejected claims. Claims 1 and 6 each have four steps: (1) hybridization of overlapping probes to a target nucleic acid; (2) determining the melting temperature of those probes and the target nucleic acid; (3) determining the difference between the melting temperature of the probes and the target and the melting temperature of the probes and a control nucleic acid (the difference is termed "ΔT_m"); (4) determining the difference between the ΔT_m of two overlapping probes. As pointed out by the Examiner, Guo *et al.* does not disclose the use of overlapping probes. Accordingly, Guo *et al.* cannot teach step (4) as described above. Southern *et al.* does not remedy this deficiency. Southern *et al.* does not teach or suggest determining the difference in ΔT_m of two overlapping probes, it merely discloses comparing the melting temperatures of probes and target nucleic acids. Accordingly, the Examiner has not carried her burden in establishing a *prima facie* case of obviousness and the rejection under 35 U.S.C. 103(a) should be withdrawn.

CONCLUSION

Applicants respectfully submit that the above remarks overcome the pending rejections under 35 U.S.C. 112, second paragraph, and 35 U.S.C. 103(a). Therefore all claims are now in condition for allowance and an early notification of such is solicited. If, upon review, the Examiner feels there are additional outstanding issues, she is invited to call the undersigned attorney. This paper is filed under 37 C.F.R. section 1.34(a).

Respectfully submitted
DORSEY & WHITNEY LLP

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By _____
Richard F. Trecartin
Reg. No. 31,801

Four Embarcadero Center, Suite 3400
San Francisco, California 94111-4187
Telephone: (415) 781-1989

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